APPROVAL SHEET

Title of Thesis: Nematodes associated with roses and the root injury caused by Meloidogyne hapla Chitwood 1949, Xiphinema diversicaudatum (Micoletzky 1927) Thorne 1939, and Helicotylenchus nannus Steiner 1945.

Name of Candidate: Ronald Allan Davis
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Thesis and Abstract Approved: W. R. Jenkins

W. R. Jenkins
Assistant Professor
Department of Botany

Date approved: May 13, 1959

NEMATODES ASSOCIATED WITH ROSES AND THE ROOT INJURY CAUSED BY *MELOIDOGYNE HAPLA* CHITWOOD 1949, *XIPHINEMA DIVERSICAUDATUM* (MICOLETZKY 1927) THORNE 1939, AND *HELICOTYLENCHUS NANNUS* STEINER 1945.

by

Ronald Allan Davis

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INTRODUCTION

Few papers have been published concerning the cytological and histological effects of plant parasitic nematodes on their hosts. Most of this type of work has been done on the root-knot disease. Christie (2) described the development of root-knot nematode incited galls on tomato seedlings, reporting that these nematodes caused hypertrophy of cortical, pericyclic, and endodermal cells, hyperplasia of the pericycle, formation of xylem elements from parenchyma surrounding giant cells, and retardation of meristematic activity in the root tip. He also reported on the development and morphology of giant cells (large, multinucleate cells resulting from a stimulatory effect of nematode feeding). Krusberg and Neilsen (8) observed similar cytological responses in their work with Meloidogyne incognita acrita Chitwood 1949 infections of Porto Rico variety of sweet potato.

Other investigators worked primarily on the cytology and morphology of giant cells and giant cell nuclei. According to Tischler (from Christie, 2), division of giant cell nuclei was by normal mitosis in early stages of giant cell development, but later divisions occurred by amitosis and by fragmentation. However, Nemec (from Christie, 2) felt that divisions by amitosis and fragmentation as reported by Tischler were actually stages of nuclear coalescence.

Linford (9) described the method by which root-knot nematodes feed on giant cells and noted that substances were extruded from the stylet during feeding. Kostoff and Kendall (7), working with galled roots of Nicotiana

hybrids, reported that secretions by the nematode increased cell wall permeability causing exosmosis and resulting in an accumulation of food in the region of invasion. Consequently, growth of plant tissues in these regions was accelerated and was expressed morphologically as swellings or galls on the roots. In 1942, Alstatt (1) tested the susceptibility of several strains and varieties of rose stocks, including Rosa multiflora Thunb. to a root-knot nematode. Of 13 different understocks, only one was found resistant. Lyle (10) and Massey (12) indicate that root-knot nematodes cause a serious disease of rose. Reynolds (15) found that in Meloidogyne incognita (Kofoid and White 1919) Chitwood 1949 infections of R. multiflora seedlings, the nematode entered the root and stimulated giant cell development; but galls occurred only rarely and were sometimes found on the end of long roots as a result of the penetration of many larvae. Martin (11) reported M. hapla as producing small, hard, galls on rose roots in Rhodesia and Nyasaland. M. hapla was reported by Van Der Linde (22) to infest a rose thornless understock.

Two genera of ectoparasitic nematodes have been associated with root gall formation. Van Gundy (23) reported that galls induced on rough lemon roots by Hemicycliophora arenaria Raski 1958 were due to a hyperplastic response of the cortical tissue. Schindler (18) demonstrated that galling of rose roots was caused by Xiphinema diversicaudatum (Micoletzky 1927) Thorne 1939, but he did not investigate their cytological effects.

In a survey of greenhouse roses, Schindler (17) found Xiphinema and Pratylenchus to be the most widely distributed genera and to occur more frequently than any other nematodes. Other genera found were: Criconemoides, Paratylenchus, Helicotylenchus, Hemicycliophora, Belonolaimus, Trichodorus, Tylenchus, Aphelenchoides, Psilenchus, and Meloidogyne.

Sher (21) described the pathogenicity of Pratylenchus vulnus Allen and Jensen 1951 on rose, reporting that rose plants infested with this species were stunted and chlorotic and the root systems were necrotic with few feeder roots. Other nematodes which have been found associated with rose are Pratylenchus pratensis (de Man 1880) Filipjev 1936 (3,14), P. penetrans (14) Sher and Allen 1953 P. scribneri Steiner 1943 (13), and Ditylenchus dipsaci (Kuhn 1857) Filipjev 1936 (5).

This present study was initiated to determine the occurrence and distribution of nematodes associated with roses grown outdoors. In addition the cytological and histological effects of Meloidogyne hapla Chitwood 1949, Xiphinema diversicaudatum (Micoletzky 1927) Thorne 1939, and Helicotylenchus nannus Steiner 1945 on rose roots was determined.

MATERIALS AND METHODS

Survey.--Soil samples were examined during the period June to October, 1958 to determine the occurrence and distribution of plant-parasitic nematodes associated with roses grown outdoors. Letters were sent to members of the American Rose Society in 27 states and Washington, D. C. requesting soil and root samples and including instructions for the proper collection of these samples. One pint of soil from each of 61 samples received was processed in a Seinhorst extraction apparatus (20). The water-soil suspension thus obtained was passed through 100 and 270 mesh screens, the residue washed off with a slow stream of water, and the resultant suspension further separated and concentrated through use of a modified Baermann funnel apparatus. Nematodes and water were drawn off into a syracuse dish after 15-20 hours and heat relaxed in an oven held at 48°C. Nematodes were then mounted in warm FAA and identified.

Source of material for cytological study.--To obtain galls produced by Meloidogyne hapla Chitwood 1949, two Rosa multiflora Thunb. seedlings were planted in 4-inch-diameter clay pots containing soil infested with this species. Root-knot galls caused by M. hapla were also obtained from naturally infected multiflora seedlings from a commercial nursery.

Galls produced by Xiphinema diversicaudatum (Micoletzky 1927) Thorne 1939 were obtained from rose, variety Better Times, which had been inoculated with specimens of this species.

Inoculation with Helicotylenchus nannus Steiner 1945 was made with specimens obtained from populations maintained in the greenhouse on tomato.

Extraction and concentration of nematodes was accomplished by the method previously described. The nematode-water suspension thus obtained was brought to a known volume and 3, 1cc. aliquots were pipetted off, placed in individual syracuse dishes, and counted. The total number of nematodes present was then approximated. Four-week old R. multiflora cuttings which had been rooted in sand were placed in steamed clay pots half full of steamed soil. Aliquots of the nematode suspension (2,150 specimens per pot) were then pipetted directly onto the roots and the pots filled with soil.

All plants in these experiments were held at a night temperature of 65-70°F. Roots of inoculated and check plants were washed free of soil and compared under a stereoscopic microscope to determine external symptoms of injury.

Preparation of slides.--Several portions of roots from plants inoculated with M. hapla, X. diversicaudatum, and H. nannus and from check plants were fixed in FAA, dehydrated by the tertiary butyl alcohol method (6), infiltrated with paraffin, and imbedded. Sections were cut at 15 micron thicknesses with a microtome and fixed to slides with Haupt's adhesive. Sections were then stained with safranin and fast green and mounted in balsam according to standard methods.

RESULTS AND DISCUSSION

Survey.--The number of soil and root samples received from each state were as follows:

STATE	NO. OF SAMPLES	STATE	NO. OF SAMPLES
California	1	New Jersey	4
Georgia	1	New York	5
Illinois	2	North Carolina	2
Indiana	2	North Dakota	1
Iowa	4	Ohio	1
Kansas	2	Pennsylvania	4
Kentucky	2	Rhode Island	1
Louisiana	1	South Carolina	1
Maryland	8	Tennessee	2
Michigan	1	Texas	1
Minnesota	1	Utah	1
Missouri	1	Virginia	7
Nebraska	1	West Virginia	2
New Hampshire	1	Washington, D. C.	1

All 61 samples contained known and possible plant-parasitic nematodes. Their occurrence is given in Table 1.

Most commonly found were the genera Xiphinema, occurring 42 times; Pratylenchus, 41 times; Helicotylenchus, 37 times; Tylenchorhynchus, 29 times; and Tylenchus, 28 times. These are substantially the same results

Table 1. Nematodes associated with rose roots in 61 samples collected from 27 states

Genus and Species	Number of Occurrences	Genus and Species	Number of Occurrences	Genus and Species	Number of Occurrences
Aphelenchoides spp.	8	Longidorus sp.	1	Rotylenchus robustus	3
A. tenuicaudatus	1				
		Meloidogyne hapla	7	Trichodorus spp.	6
Aphelenchus spp.	8				
		Neotylenchidae	20	Tylenchorhynchus sp.	1
Belonolaimus gracilis	1			T. brevidens	8
		Paratylenchus spp.	7	T. claytoni	13
Criconemoides spp.	4	P. dianthus	3	T. cylindricus	1
		P. projectus	8	T. dubius	4
Ditylenchus sp.	1			T. nudus	1
		Pratylenchus penetrans	26	T. parvus	1
Gottholdsteineria buxophila	1				
		P. pratensis	7	Tylenchus spp.	28
Helicotylenchus multicinctus	1	P. scribneri	1		
		P. thornei	1	Xiphinema americanum	41
H. nannus	34	P. vulnus	6	X. krugi	1
Hemicycliophora spp.	8	Psilenchus spp.	4		
Hoplolaimus tylenchiformis	6				

as those reported by Schindler (17). The most commonly found species was Xiphinema americanum Cobb 1913, occurring in 41 samples. Helicotylenchus nannus Steiner 1945, the next most common species, occurred 34 times; Pratylenchus penetrans (Cobb 1917) Chitwood and Oteifa 1952, occurred 26 times; and Tylenchorhynchus claytoni Steiner 1937, occurred 13 times. Many of these genera and species are known pathogens on other hosts and it is therefore probable that they may also cause diseases of roses.

Effects of Meloidogyne hapla.--A root system heavily infected with M. hapla is shown in Fig. 1. Sectioned and stained galls showed the presence of individuals of this nematode within the cortex, stele, and root tip (Figs. 2,3,4,6,9,18). For comparison, cross sections of uninoculated roots are illustrated in Figs. 5 and 17. Vascular tissue was apparently the preferred feeding site since most nematodes were observed lying with their anterior ends imbedded within this tissue with their bodies extending into the cortex (Figs. 2,3,4). Females deposited their eggs near the surface of the root (Figs. 3,18). In some infected root tips, the presence of the parasite apparently suppressed mitotic activity in the apical meristem and growth was retarded (Fig. 2). Hyperplasia of the cortex and vascular parenchyma (Fig. 4) and giant cell formation were observed to accompany all infections. Giant cells formed around the anterior end of the nematode (Figs. 2,3,4,6,9) and were generally located in the vascular tissue or root tip. These cells were less often observed in the cortex, a development similar to that described by Christie (2).

Giant cell formation in differentiated tissues began with the enclosing of a group of vascular parenchyma cells by a thick wall. Walls

of the enclosed cells then disintegrated, protoplasmic contents coalesced, and a giant cell resulted (Figs. 7,8). This type of giant cell development has not been previously reported in the literature and may be specific for this host.

In undifferentiated tissues of the root tip, giant cells developed in the same manner as reported by Christie (2); i.e., from the dissolution of cell walls with a subsequent deposition of a thick wall around the coalesced cytoplasm and nuclei. As illustrated in Fig. 9, a giant cell of this type can be seen developing in the region of elongation. Lysis of procambial cells was evident. The cytoplasm appeared granular in all giant cells; however, the cytoplasm stained red in old giant cells; gray in young or developing giant cells. This reaction was also observed by Krusberg and Neilsen working with M. incognita acrita infections of Porto Rico variety of sweet potato (8). It is possible that the differential staining reaction is due to a chemical change which occurs in the giant cells as they grow older.

Nuclei of giant cells varied in number depending upon the region in which they were located and their age. They varied in appearance even between adjacent giant cells. Nucleoli in all giant cells were as large, or larger than normal nuclei and stained deeply. In root tips, young giant cells contained 30-40 nuclei aggregated in the center of the cell (Fig. 10), while within the stele the nuclei were less numerous and usually much larger (Figs. 11,12,13). The number of nuclei in a single giant cell may depend upon the number of cells which go into its formation. If this were the case, it would explain the increased number of nuclei in root tip giant cells since many procambial cells are involved

in their formation. Also, nuclei of older giant cells may have coalesced or disintegrated, thus reducing the number of nuclei observed. Nuclei in some giant cells appeared to be partitioned off with a nucleolus in each cavity (Fig. 11), while in other giant cells they were donut shaped and nucleoli were arranged around this ring (Fig. 12). As reported by Krusberg and Neilsen (8), and Christie (2), nuclear membranes of nuclei in young giant cells were distinct (Fig. 10), while nuclear membranes of nuclei in some older giant cells were indistinct and appeared to be disintegrating (Fig. 13). In some infections the nuclei of cortical cells surrounding the nematode body and the nuclei of those cells around the newly formed giant cells were increased approximately 2-3 times in size (Figs. 4,9). As reported by other investigators (2,8), xylem elements appeared to form from xylem parenchyma around some giant cells. These cells had no definite arrangement or shape and were short, reticulate elements (Fig. 14).

Longitudinal sections through galled roots revealed that giant cells in the vascular cylinder interrupted the continuity of some vessels and other vascular tissues (Fig. 15). The interruption of these stelar tissues undoubtedly would have an effect on translocation of water and nutrients through the roots and could account for much of the injury resulting from root-knot infection. When roots in which a considerable amount of secondary growth had occurred were attacked, giant cells formed but were smaller and less numerous than giant cells formed in younger roots. In these cases, the anterior end of the nematode became imbedded in the stele and the posterior portion in the periderm. Peridermal tissues proliferated around the body of the nematode and a layer of cork was formed around the anterior end of the nematode (Fig. 16). Figure 17 is a photo-

micrograph of a cross section of a non-infected root of the same age.

When multiple infection of single primary roots occurred, roots became rough and increased greatly in diameter. As many as 12 females and egg masses imbedded in a single cross section were observed (Fig. 18). Large cavities occurred in the center of the root, probably due to collapse of giant cells. It is evident that the translocation of water and nutrients in roots of this condition would be greatly impaired if not entirely stopped.

Figure 1. *Rosa multiflora* root system heavily infected with *Meloidogyne hapla*. Many small root galls are evident.

Figure 2. Longitudinal section through a *Rosa multiflora* root tip gall infected with *Meloidogyne hapla* showing a swollen female and several giant cells. Meristematic activity in the apical meristem has ceased (X190).

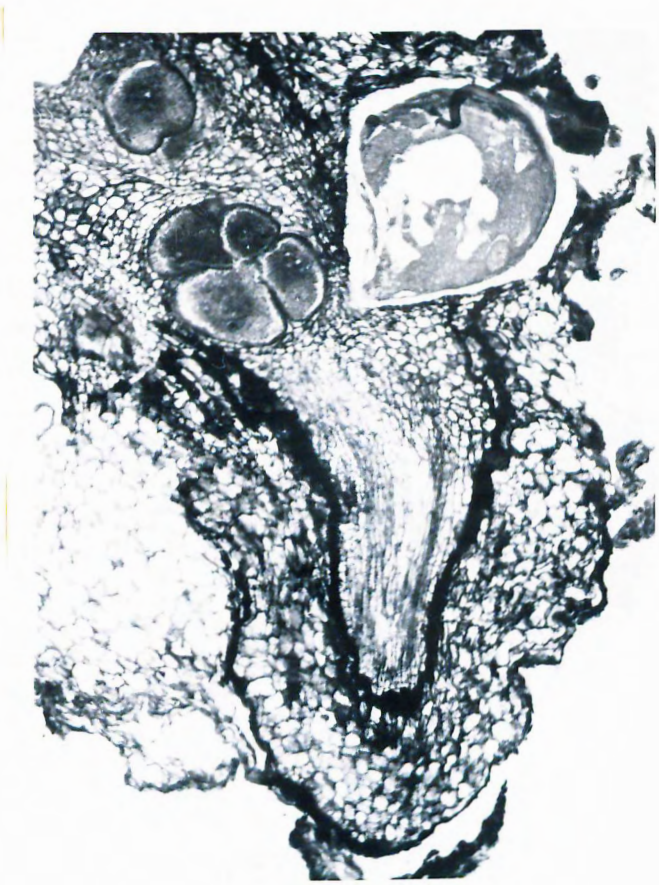


Figure 3. Cross section of a root-knot gall on Rosa multiflora showing a Meloidogyne hapla female and an accumulated egg mass near the surface of the root (X190).

Figure 4. Cross section through a galled root of Rosa multiflora. Note the large nuclei of proliferated cortical cells (arrow) on one side of the nematode body. Vascular and cortical hyperplasia is evident. At the anterior end of the nematode, a giant cell and two groups of vascular parenchyma cells are enclosed by thick walls (X190).

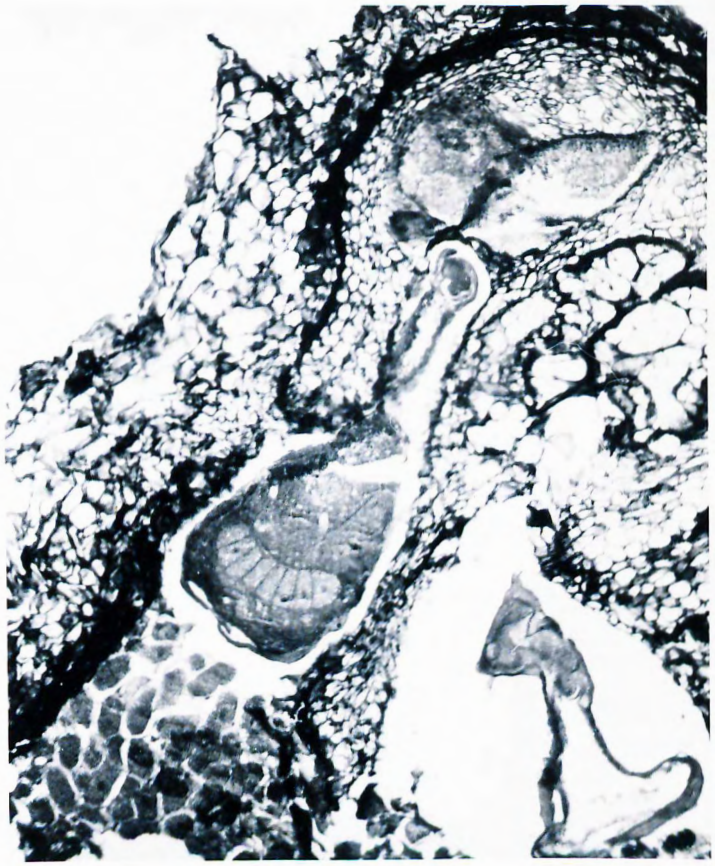
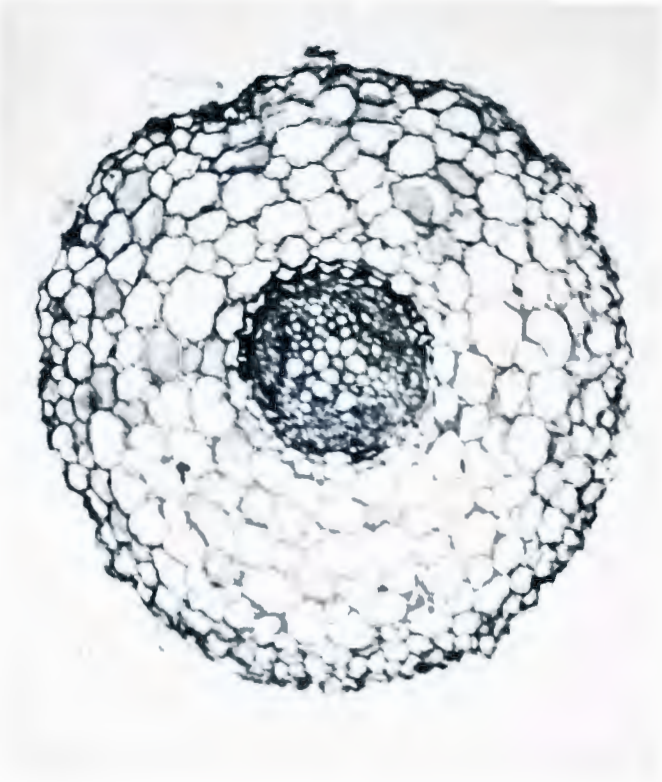
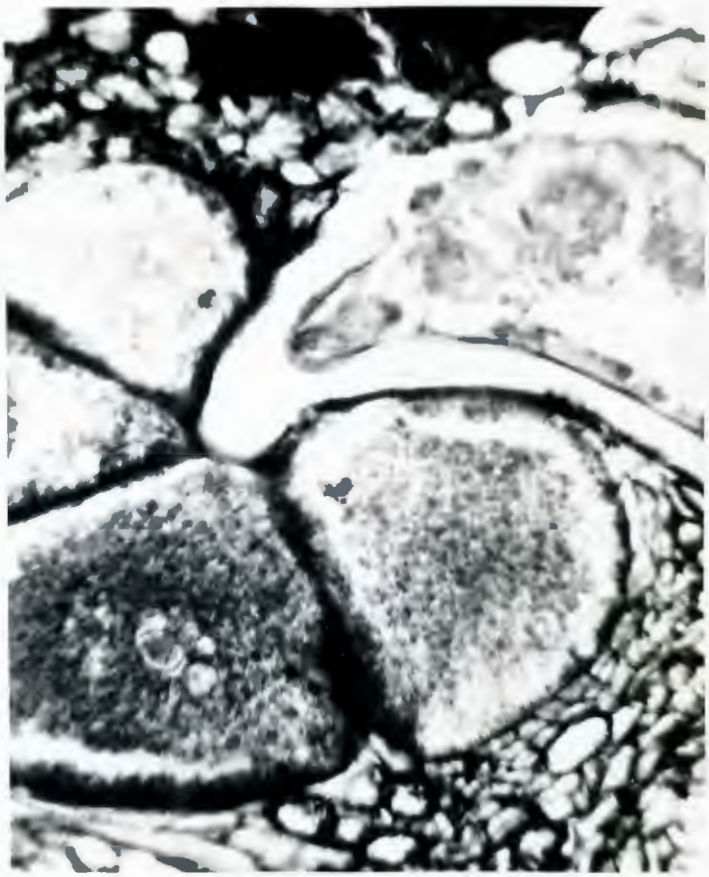


Figure 5. Cross section of an uninoculated Rosa multiflora root approximately the same age as those roots illustrated in Figures 3 and 4 (X95).

Figure 6. Cross section through a Rosa multiflora root gall incited by Meloidogyne hapla showing a group of 4 giant cells clustered about the anterior end of the nematode (X500).





Figures 7 and 8. Longitudinal serial sections through a Rosa multiflora root gall infected with Meloidogyne hapla illustrating giant cell development in differentiated tissues. In Figure 7, a group of vascular parencyma cells are enclosed by a thick wall. Serial sections revealed that the cell walls of these enclosed parenchyma cells disintegrated, their protoplasmic contents coalesced, and a giant cell developed as illustrated in Figure 8 (X370).

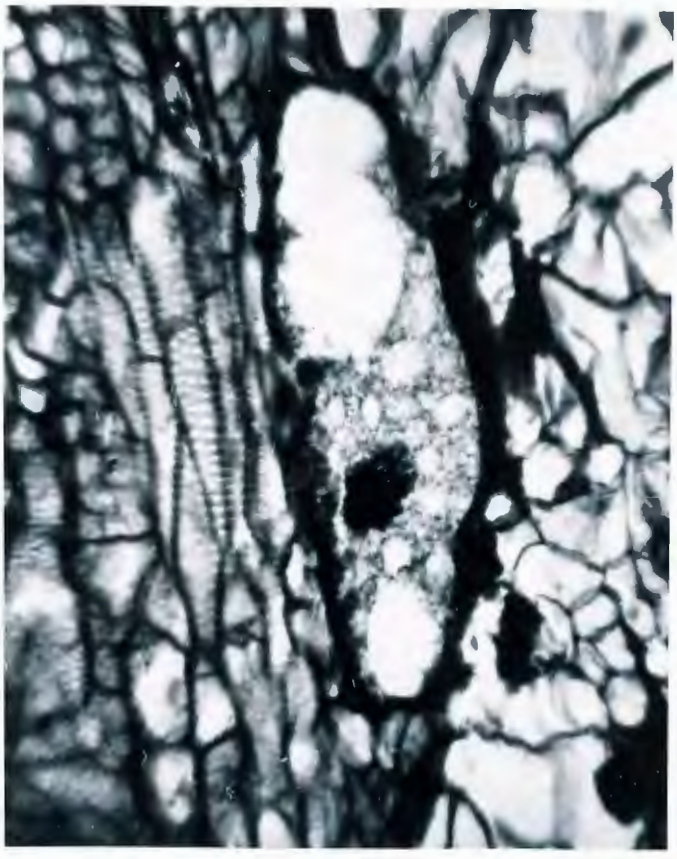
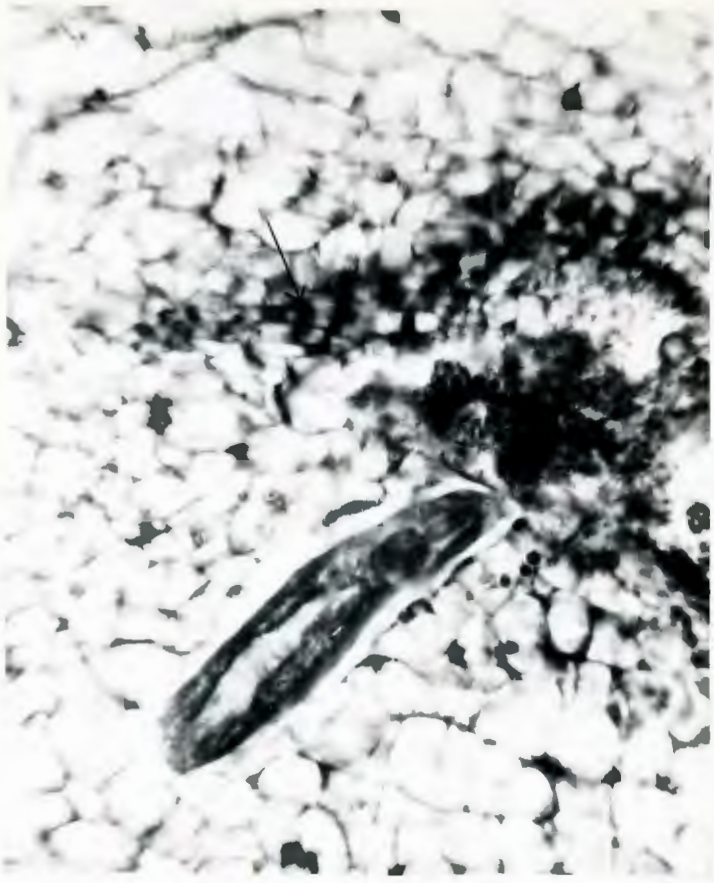


Figure 9. Longitudinal section through Rosa multiflora root tip showing a Meloidogyne hapla larva, imbedded in the region of elongation, and a developing giant cell. Note lysis of procambial cells bordering the giant cell and the large nuclei of some of these cells (arrow) (X400).

Figure 10. Longitudinal section through Rosa multiflora root tip infected with Meloidogyne hapla showing many nuclei aggregated in the center of a young giant cell (X1200).



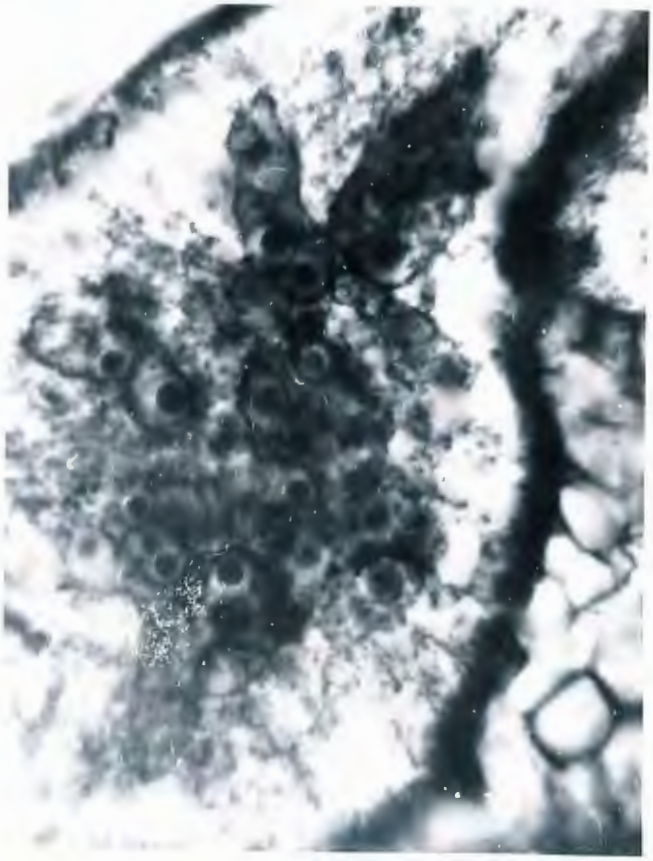
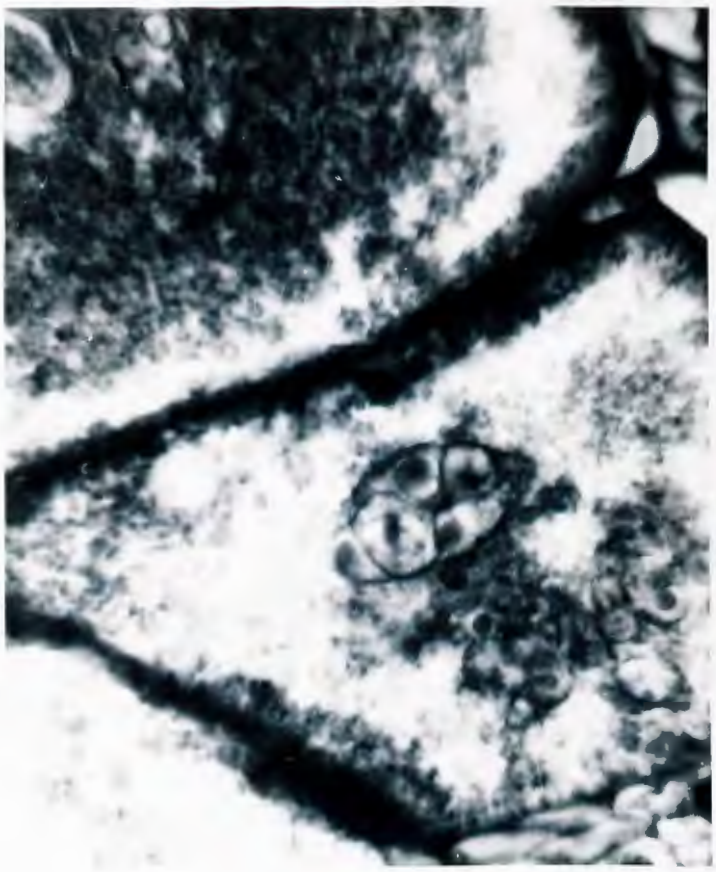


Figure 11. Cross section through a Rosa multiflora root tip gall infected with Meloidogyne hapla showing a giant cell nucleus which appears to be partitioned off into sectors, each containing a nucleolus (X780).

Figure 12. Cross section through a Rosa multiflora root gall infected with Meloidogyne hapla illustrating a donut shaped giant cell nucleus in which nucleoli can be observed around the ring (X1750).



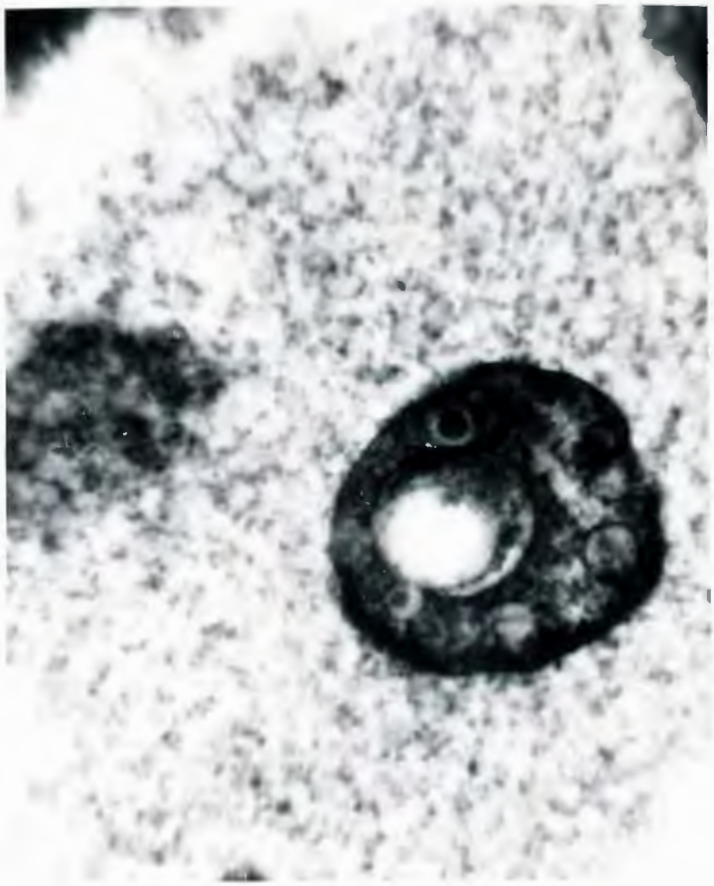
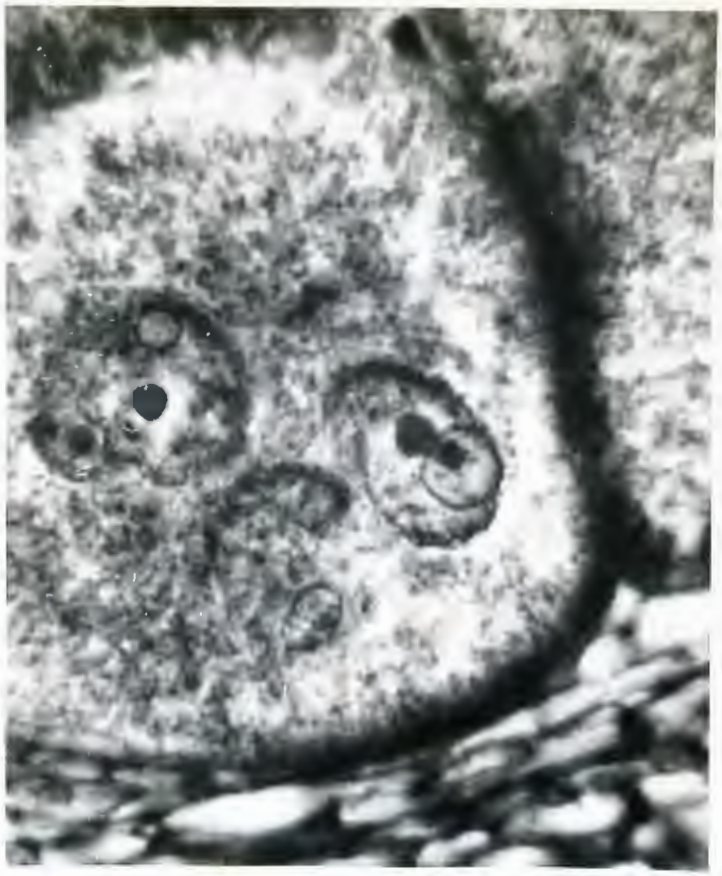


Figure 13. Cross section through a Rosa multiflora root gall infected with Meloidogyne hapla illustrating the disintegration of nuclear membranes of nuclei in a giant cell (X1100).

Figure 14. Longitudinal section through a Rosa multiflora root gall infected with Meloidogyne hapla illustrating the short, reticulate, xylem elements (arrow) which have formed from xylem parenchyma (X530).



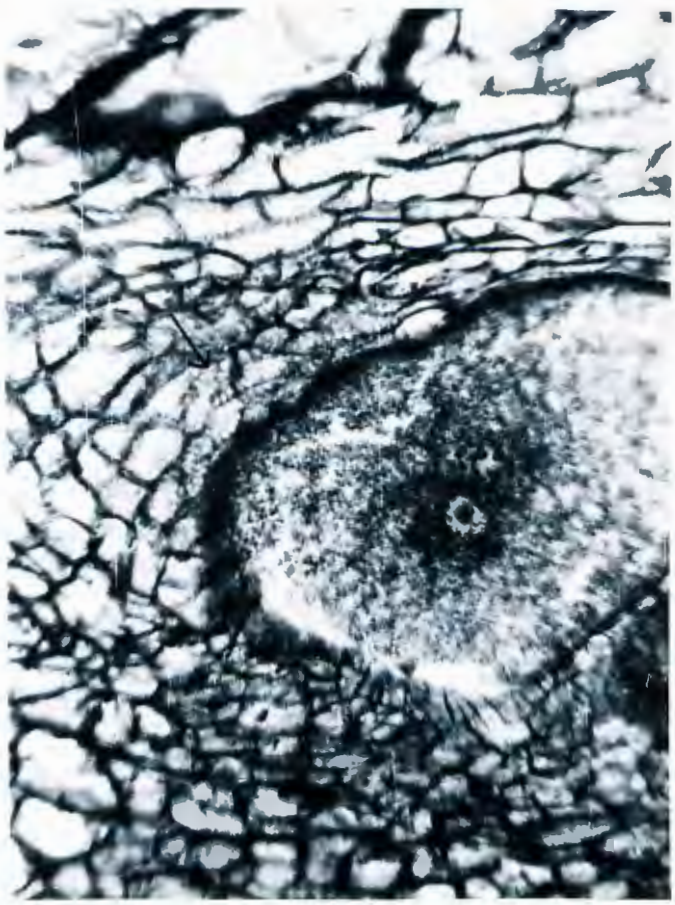
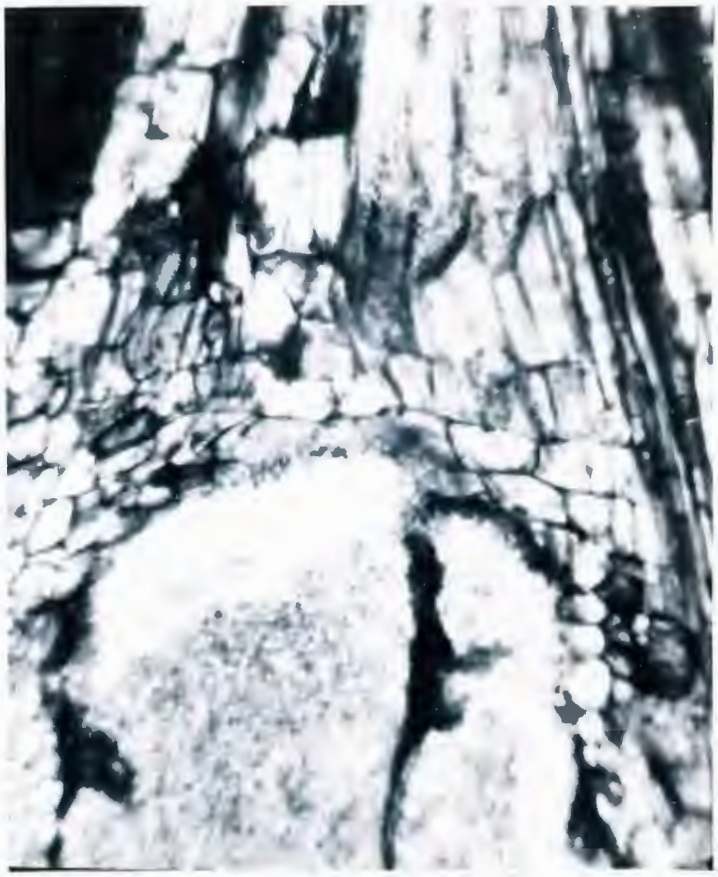


Figure 15. Longitudinal section through a Rosa multiflora root gall infected with Meloidogyne hapla showing interruption of vessels by giant cells (X575).

Figure 16. Cross section of an older Rosa multiflora root attacked by Meloidogyne hapla. Note cork formation around cavity left by the anterior end of the nematode and the proliferated peridermal tissues which enclosed the posterior portion of the nematode (X100).



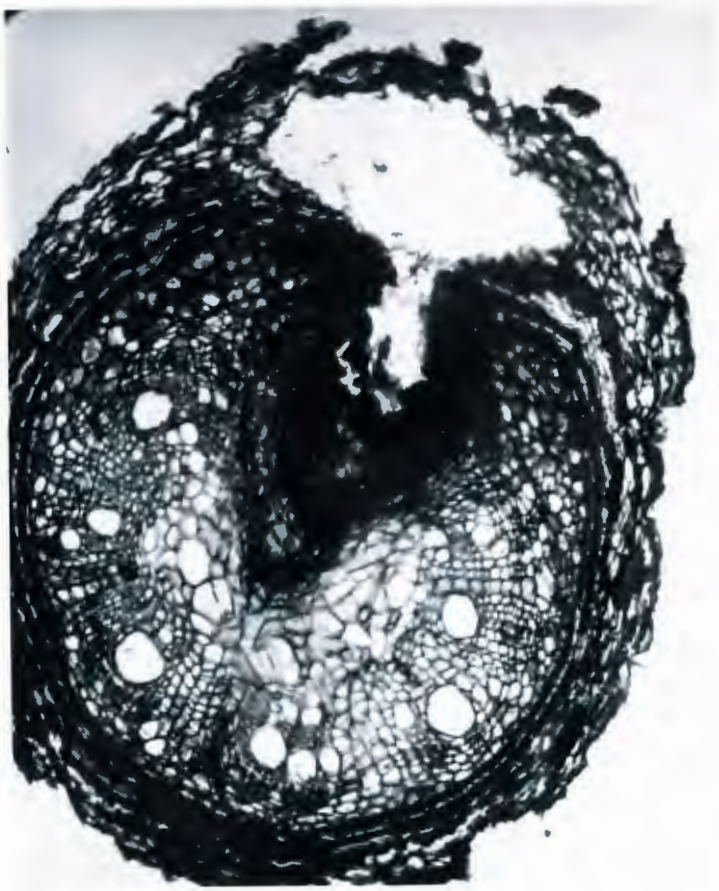
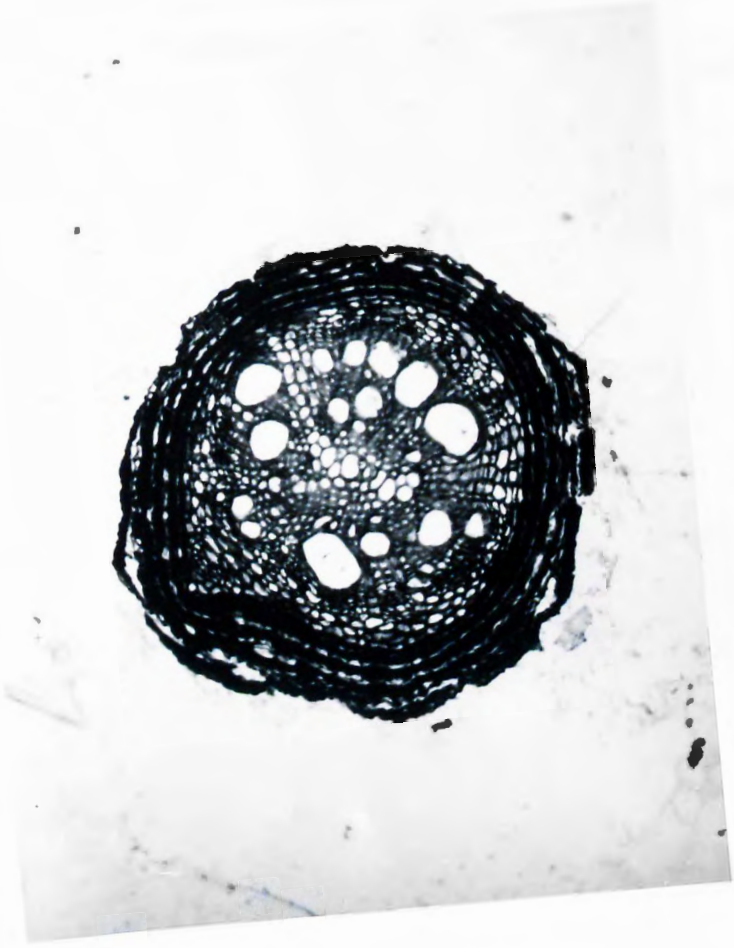
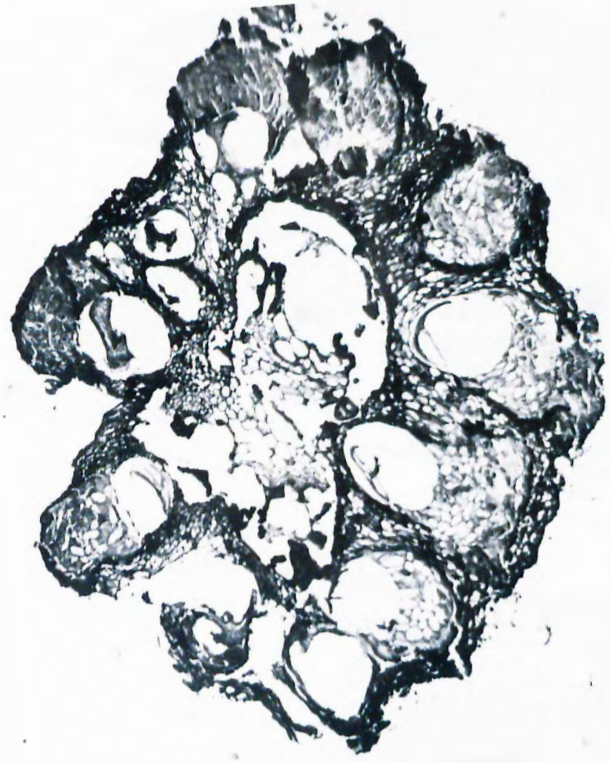


Figure 17. Cross section of uninfected Rosa multiflora root of approximately the same age as infected root illustrated in Figure 16 (X100).

Figure 18. Cross section of Rosa multiflora root showing 12 or more Meloidogyne hapla females and egg masses imbedded in the root. Large cavities in the center of the root are probably due to collapse of giant cells (X40).





Effects of Xiphinema diversicaudatum.--Microscopic examination of sectioned and stained galled roots revealed cytological and histological abnormalities when compared with ungalled control roots (Figs. 19-27). Gall formation was due primarily to a hyperplastic response of the cortical tissue. Cortical cells of swollen and curled infected roots showed hyperplasia on the inside of the curve. These cells proliferated to form 15-20 cell layers more than the opposite side of the root (Fig. 19). In root tips which were swollen but not curled, cortical hyperplasia occurred evenly around the root. The epidermis at infection sites was broken and cortical cells beneath the break stained red (Figs. 21,22). Check roots showed normal development (Figs. 20,26).

Cortical cells associated with some infection sites increased in size approximately 2-3 times, their walls thickened, and their cytoplasm appeared granular (Fig. 23). These cells were similar in appearance to giant cells produced by root-knot nematodes, except that nuclei exhibited only slight hypertrophy and only 2-3 nuclei were observed within the cell. In some cases, cortical cells lying adjacent to or very near these giant cells contained deeply red staining, spherical bodies which appeared to be attached to the cell wall (Fig. 24). These bodies were sometimes observed clustered in the lumen of the cell (Fig. 25). The origin and composition of these structures is not known.

Meristematic activity in infected root tips was retarded but the degree of retardation varied from root to root. Apical meristems of some galled root tips did not stain as deeply as the meristematic regions of control root tips and there were no root caps, however, vascular differentiation appeared normal (Figs. 19,26). In other parasitized roots, the apical meristem was reduced in size and in some cases was almost lacking

(Fig. 27). Vascular differentiation in such roots extended far down into the root tip, an effect reported by other investigators (16,19).

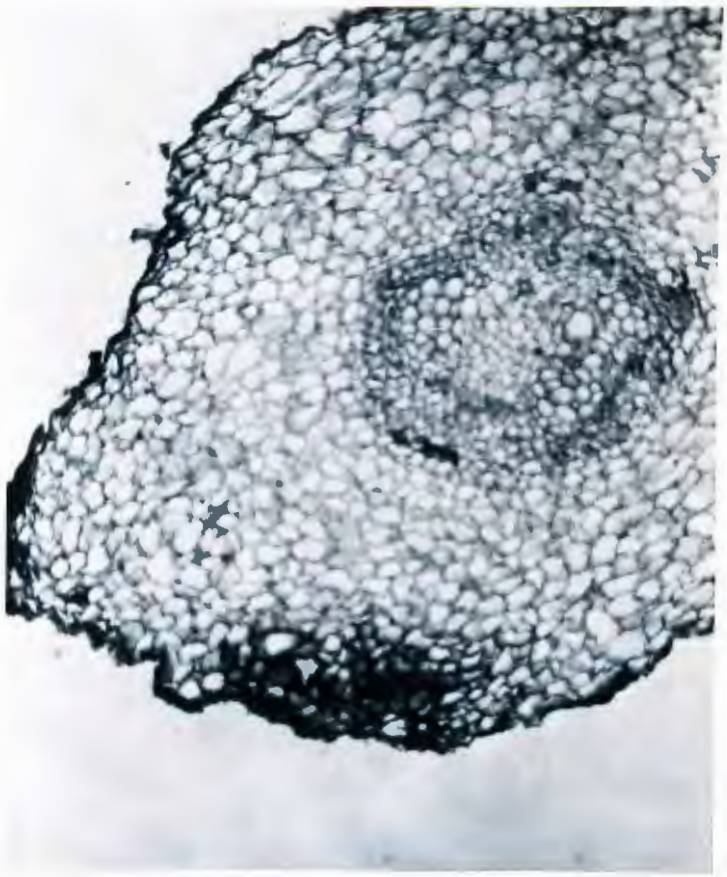
Injury caused by X. diversicaudatum was similar in some respects to that caused by root-knot nematodes in that feeding by the parasite stimulated cortical hyperplasia, retardation of meristematic activity in the root tip, and giant cell formation. Xiphinema injury differed in that it was confined to the root tip, epidermis, and cortex; cells of the epidermis and cortex were ruptured when the nematode entered the root; and cortical cells associated with some infections contained red staining, spherical bodies.

Figure 19. Longitudinal section through a rose root gall produced by Xiphinema diversicaudatum. Note the proliferated cortical tissue on the inside of the curve, and the absence of a root cap (X34).

Figure 20. Cross section through an uninoculated rose root illustrating normal development (X100).

Figure 21. Cross section through a rose root gall incited by Xiphinema diversicaudatum illustrating injury to cortical tissue at a single infection site (X100).

Figure 22. Cross sectional view of Xiphinema diversicaudatum injury site. Note the broken epidermis and the dark, granular nature of cortical cells beneath the rupture (X435).



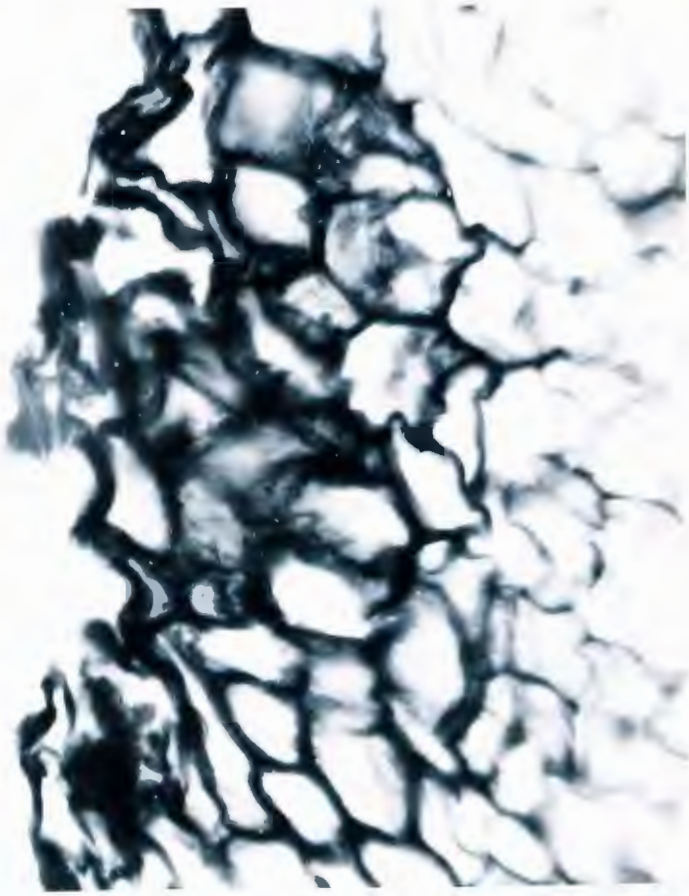
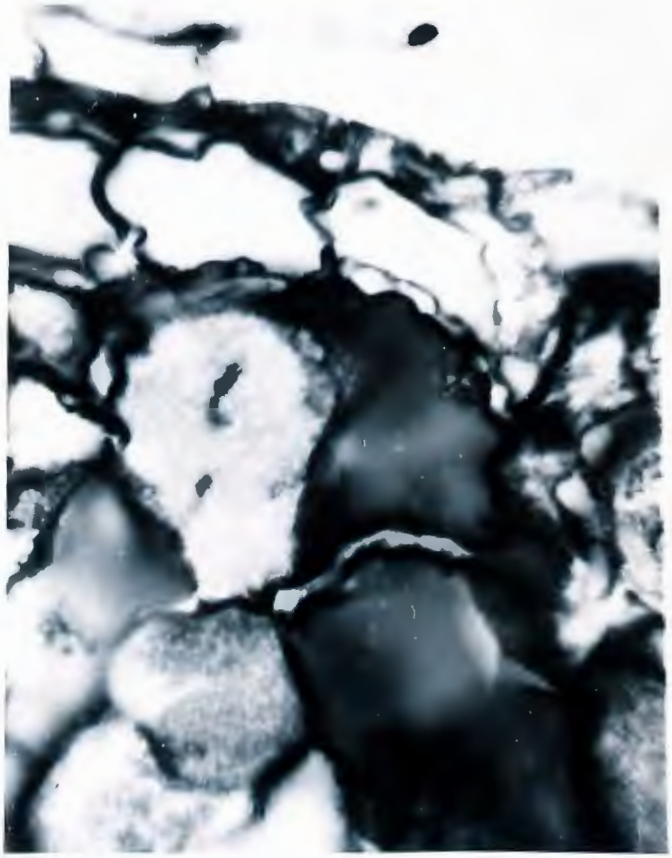


Figure 23. Cross section through a rose root infected with Xiphinema diversicaudatum illustrating giant cells which have developed as the result of nematode feeding. Note the thick walls and granular nature of the cytoplasm (X1000).

Figure 24. Longitudinal section through a rose root infested with Xiphinema diversicaudatum illustrating deep staining, spherical structures adhering to the walls of a cortical cell (X1000).



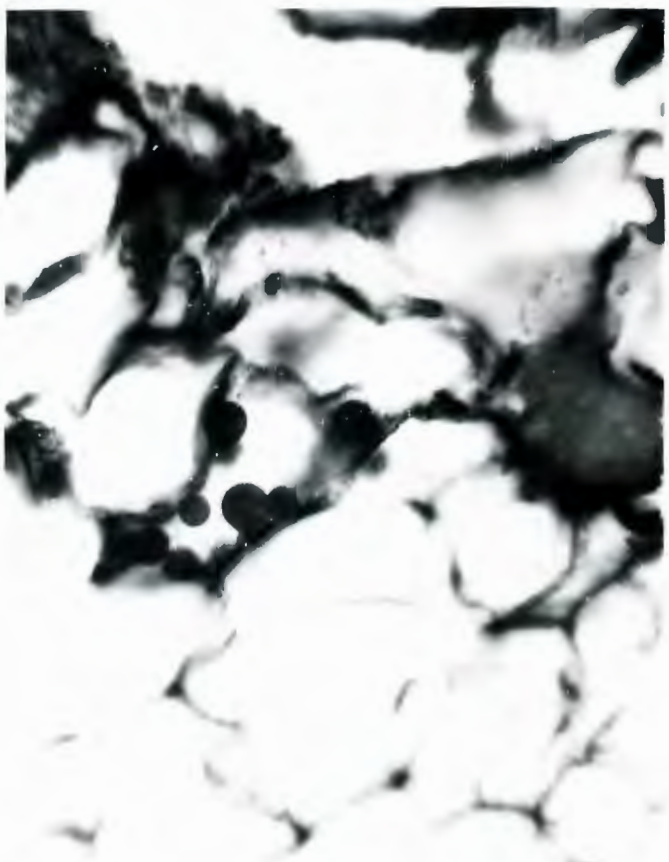


Figure 25. Iongitudinal section through a rose root infested with Xiphinema diversicaudatum illustrating dark staining, spherical structures clustered in the lumen of a cortical cell. Several surrounding cells contain granular cytoplasm (X900).

Figure 26. Iongitudinal section through an uninoculated rose root tip illustrating normal development (X105).

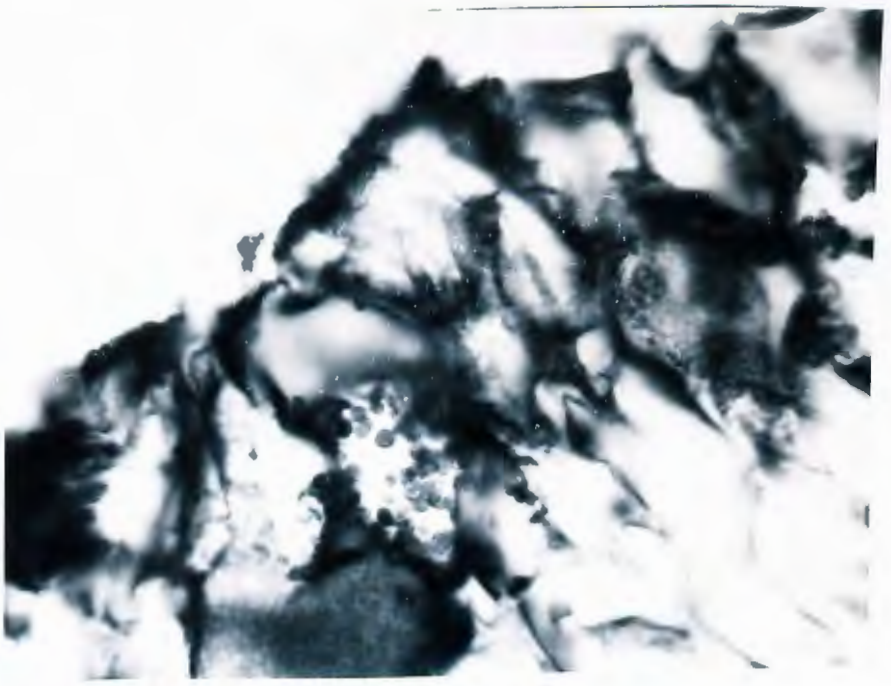
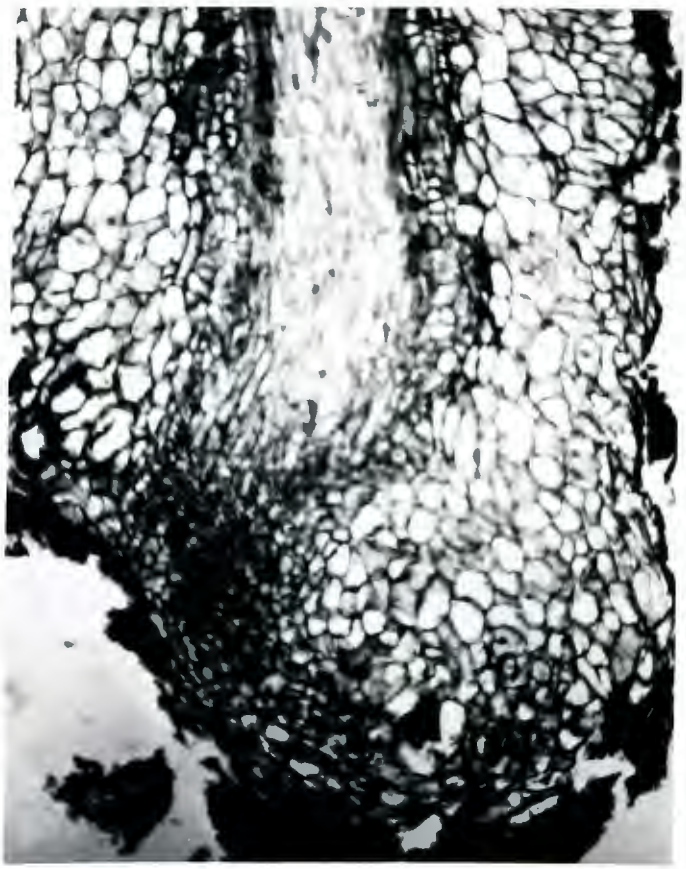


Figure 27. Longitudinal section through a rose root tip gall incited by *Xiphinema diversicaudatum*. Note the abnormal meristematic region and hyperplasia of surrounding cells. Vascular differentiation has extended far down into the root tip (X105).

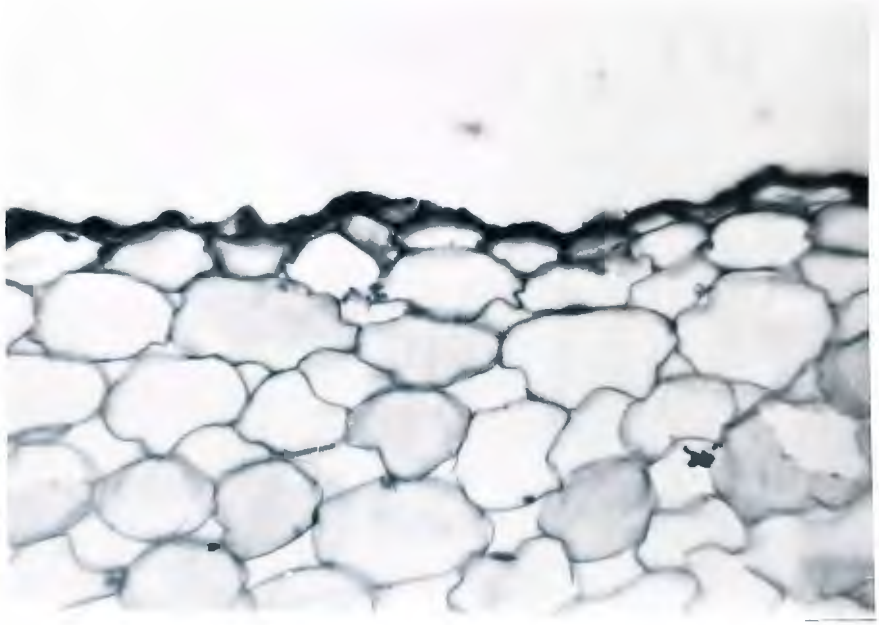


Effects of Helicotylenchus nannus.--Microscopic examination of infected whole roots revealed that roots of 1 mm. in diameter or less were most heavily attacked. Infected roots were marked by tiny, brown necrotic lesions similar to those described on boxwood by Golden (4). More often, infected roots showed tiny, brown, discolored areas rather than lesions. Sectioned and stained material revealed that the surface of infected roots was broken in some spots and several cortical cells beneath the lesion assumed a deep green stain (Fig. 29). In other roots, where no lesions occurred, cortical cells directly beneath the epidermis stained deep red to black (Fig. 30), indicating a chemical change in cell contents stimulated by nematode feeding or injection of chemical substances. These affected cortical cells sometimes extended several cell layers into the root, suggesting that the stimulant diffuses into adjacent cells. The epidermis was not affected, therefore, the stylet of the nematode either pierced the epidermis or went between the epidermal cells while the nematode fed on the cortical cells directly beneath. No lesions or necrotic areas were observed on the roots of control plants (Fig. 28). The histopathology of H. nannus on rose was similar to that of Gottholdsteineria buxophila on boxwood (4). However, lesions did not extend as deeply into the cortex, and in some roots, cortical cells directly beneath the epidermis were affected without apparent injury to the epidermis.

H. nannus is obviously pathogenic to rose, causing direct injury by feeding on the roots. This feeding results in cell destruction and a devitalization of roots through the removal of cell contents.

Figure 28. Cross sectional view of uninoculated Rosa multiflora root showing normal development (X575).

Figure 29. Cross section of Rosa multiflora root attacked by Helicotylenchus nannus. Note broken surface and lesion extending into the cortex. Several cortical cells beneath the lesion are deeply stained (X875).



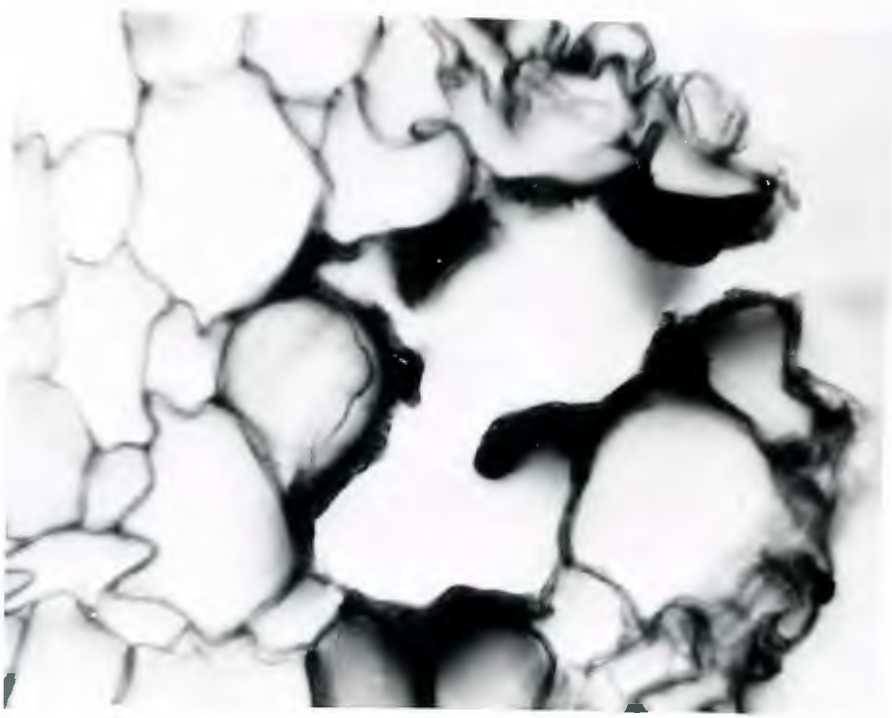
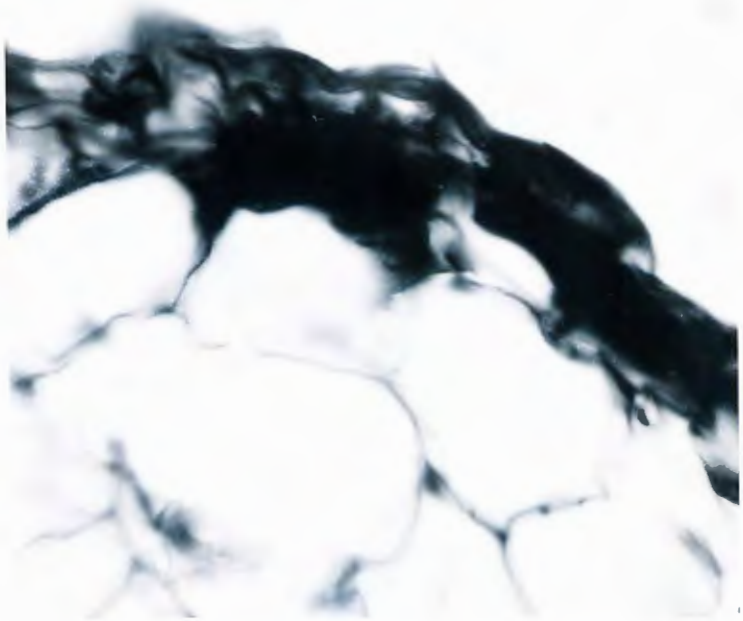


Figure 30. Cross section of Rosa multiflora root parasitized by Helicotylenchus nannus illustrating affected cortical cells beneath the epidermis. Note that the epidermis is apparently uninjured (X930).



SUMMARY

All samples examined in a survey of nematodes associated with roses grown outdoors contained plant parasitic forms. These forms included the genera Belonolaimus, Criconemoides, Helicotylenchus, Hemicycliophora, Hoplolaimus, Longidorus, Meloidogyne, Paratylenchus, Pratylenchus, Rotylenchus, Trichodorus, Tylenchorhynchus, and Xiphinema.

Sectioned and stained root-knot galls from rose roots showed the presence of Meloidogyne hapla within the root causing giant cells, hyperplasia of the cortical and vascular parenchyma, xylem elements formed from vascular parenchyma, and retardation of meristematic activity in the root tip. Giant cells in differentiated tissues were formed from a group of vascular parenchyma cells which were first enclosed by thick walls. Walls of enclosed parenchyma cells disintegrated, the protoplasmic contents coalesced, and a giant cell developed. In undifferentiated tissues, giant cells formed from the dissolution of cell walls with a subsequent deposition of a thick wall around the coalesced cytoplasm and nuclei. Giant cell nuclei varied in size and appearance.

Sectioned and stained root galls incited by Xiphinema diversicaudatum revealed that gall formation was due primarily to a hyperplastic response of cortical cells. Cortical cells associated with some feeding sites increased 2-3 times in size, their cytoplasm appeared granular, and their walls thickened. In some infections, cortical cells lying adjacent to, or very near, these giant cells contained spherical structures adhering to the walls or clustered in the lumen. Meristematic activity in infested

root tips was retarded and vascular differentiation extended far into the root tip.

Rose roots parasitized by Helicotylenchus nannus were marked by tiny brown necrotic lesions and discolored areas. Sections revealed that the surface of infected roots was broken and several cortical cells beneath the lesion stained deeply. Where no lesions occurred, cortical cells directly beneath the epidermis were affected without apparent injury to the epidermis.

Much of the injury caused by Meloidogyne hapla, Xiphinema diversicaudatum, and Helicotylenchus nannus is apparently due to chemical changes within root tissues, possibly resulting from nematode secretions or a host reaction to nematode feeding. The extensive root injury caused by these nematodes probably results in decreased top growth, unproductivity, and increased susceptibility to other disease agents.

LITERATURE CITED

1. Altstatt, G. E. 1942. Susceptibility of some common rose understocks to nematode root-knot. Plant Disease Reptr. 26:371.

2. Christie, J. R. 1936. The development of root-knot nematode galls. Phytopathology 26:1-22.

3. Crossman, Louise, and J. R. Christie. 1937. Lists of plants attacked by miscellaneous plant-infesting nematodes. Plant Disease Reptr. 21: 144-167.

4. Golden, A. M. 1956. Taxonomy of the spiral nematodes (Rotylenchus and Helicotylenchus), and the developmental stages and host-parasite relationships of R. buxophilus, n. sp., attacking boxwood. Univ. of Md. Agric. Expt. Sta. Bull. A-85. 28 p.

5. Goodey, J. B., and Mary T. Franklin. 1956. The nematode parasites of plants catalogued under their hosts. Commonwealth Agric. Bur., Farnham Royal, Bucks., England. 140 p.

6. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., Inc., New York, N.Y. 523 p.

7. Kostoff, D., and J. Kendall. 1930. Cytology of nematode galls on Nicotiana roots. Abst. Centralbl. Bakt. II 81:86-91.

8. Krusberg, L. R., and L. W. Nielsen. 1958. Pathogenesis of root-knot nematodes to the Porto Rico variety of sweet potato. Phytopathology 48:30-39.

9. Linford, M. B. 1937. The feeding of the root-knot nematode in root tissue and nutrient solution. Phytopathology 27:824-835.

10. Lyle, E. W. 1943. Nematode resistance in rose understocks. Am. Rose Annual 28:157-158.

11. Martin, G. C. 1954. Nematodes. Rhodesian Tobacco J. 6(6):115.

12. Massey, L. M. 1947. Soil fumigants for root-knot control. Am. Rose Annual 32:119-124.

13. Nesbitt, R. B. 1956. New host plants of plant parasitic nematodes in California. Plant Disease Reptr. 40:276.

14. Oostenbrink, M. 1956. O ver de invloed van verschillende gewassen op de vermeerdering van en de schade door Pratylenchus pratensis en Pratylenchus penetrans (Vermes, Nematoda), mit vermelding van een afwijkend moeheidsverschijnsel bij houtige gewassen. Tijdschr. Plantenziekten 62:189-203.

15. Reynolds, H. W. 1950. Root-knot nematode development and root tissue response of the rose. Ph. D. Thesis, Purdue Univ. 56 p.

16. Rohde, R. A., and W. R. Jenkins. 1957. Host range of a species of Trichodorus and its host-parasite relationships on tomato. Phytopathology 47:295-298.

17. Schindler, A. F. 1956. Nematodes associated with roses in a survey of commercial greenhouses. Plant Disease Reptr. 40:277-278.

18. Schindler, A. F. 1957. Parasitism and pathogenicity of Xiphinema diversicaudatum, an ectoparasitic nematode. Nematologica 2:25-31.

19. Scott, Marie. 1959. In plant nematodes their bionomics and control, by J. R. Christie. Agric. Expt. Sta. Fla. p 15.

20. Seinhorst, J. W. 1956. The quantitative extraction of nematodes from soil. Nematologica 1:249-267.

21. Sher, S. A. 1957. A disease of roses caused by a root-lesion nematode, Pratylenchus vulnus. Phytopathology 47:703-706.

22. Van Der Linde, W. J. 1956. The Meloidogyne problem in South Africa. Nematologica 1:177-183.

23. Van Gundy, S. D. 1958. The pathogenicity of Hemicycliophora arenaria on citrus. Phytopathology 48:399 (Abst.)

VITA

Name: Ronald Allan Davis

Permanent address: VF - 12F, College Park, Maryland

Degree and date to be conferred: Master of Science, 1959

Date of birth: December 4, 1935

Place of birth: Penns Grove, New Jersey

Secondary education: Penns Grove Regional High School, Penns Grove, New Jersey

Collegiate institutions attended	Dates	Degree	Date of Degree
University of New Hampshire	1953-1957	B.S.	June, 1957
University of Maryland	1957-1959	M.S.	June, 1959

Major: Plant Pathology (Nematology)

Minor: Botany

Publications:

1. Cytological and histological effects of Xiphinema diversicaudatum (Micoletzky, 1927) Thorne 1939 and Meloidogyne hapla Chitwood 1949 on rose roots. (Abst.) Phytopathology (In press.)

Positions held:

1. Graduate Assistant, University of Maryland. 1957 to present.